Br. J. Pharmac. (1984), **83**, 889–898

Effects of reduced calcium ion concentration and of diltiazem on vasoconstrictor responses to noradrenaline and sympathetic nerve stimulation in rat isolated tail artery

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1 In isolated, perfused proximal segments of Sprague-Dawley rat tail artery, idazoxan (100 nmol l^{-1}) displaced the concentration-response curve to noradrenaline (NA) to the right. The log shift of the NA concentration-response curve was greater at lower concentrations than at higher concentrations of NA. Idazoxan (100 nmol l^{-1}) had no effect on responses to electrical stimulation.

2 Prazosin (10 nmol l^{-1}) displaced the concentration-response curve to NA to the right as well as markedly reducing responses to sympathetic nerve stimulation.

3 The concentration-response curve to NA, obtained after reducing the concentration of calcium ions in the Krebs solution from 2.5 to 0.6 mmol l^{-1}, was significantly displaced to the right. Responses to sympathetic nerve stimulation were not affected by this reduction in the concentration of calcium ions.

4 Diltiazem (1 and 10 μmol l^{-1}) significantly displaced the concentration-response curve to NA to the right but had no effect on sympathetic nerve stimulation.

5 These *in vitro* results in peripheral arterial smooth muscle confirm the findings of previous *in vivo* studies which suggest that α_2-adrenoceptors contribute to the vasoconstrictor responses elicited by α-adrenoceptor agonists and that these responses but not those mediated by α_1-adrenoceptors are dependent on extracellular calcium.

Introduction

The subclassification of α-adrenoceptors, until recently, was based on their anatomical location. Classical postjunctional α-adrenoceptors on the smooth muscle cell membrane which mediate vasoconstriction were termed α_1-adrenoceptors; pre-junctional α-adrenoceptors which are involved in the regulation of transmitter release were termed α_2-adrenoceptors (Langer, 1974). However, the development and use of selective α-adrenoceptor agonist and antagonist drugs has led to the realization, at least from *in vivo* studies, that vascular smooth muscle α-adrenoceptors are not exclusively of the α_1-subtype (Drew & Whiting, 1979; Docherty & McGrath, 1980a; Hamilton & Reid, 1980; Langer & Shepperson, 1982; McGrath, 1982). Noradrenaline (NA) for example, was shown to produce pressor responses in the pithed rat and in the anaesthetized cat which were less susceptible to blockade by prazosin than to blockade by either phentolamine or yohimbine (Drew & Whiting, 1979). In the latter study, NA was acting through α-adrenoceptors, as indicated by the effects of phentolamine and yohimbine but these receptors were not solely of the α_1-subtype, as demonstrated by the resistance of NA responses to blockade by prazosin.

It has also been found that the effects of circulating or exogenous agonists are susceptible to blockade by both α_1- and α_2-adrenoceptor antagonists (Docherty & McGrath, 1980a, b; Flavahan & McGrath, 1980) whereas responses to sympathetic nerve stimulation (SNS) are more susceptible to blockade by α_1-adrenoceptor antagonists (Docherty & McGrath, 1980a, b). The most attractive hypothesis to explain these data is that the outer layers of well-innervated vessels contain only α_1-adrenoceptors which respond to transmitter NA released from sympathetic nerve terminals, whereas the inner layers contain both α_1-

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and α_2-subtypes which may be activated by circulating α-adrenoceptor agonists (McGrath, 1982).

There are also differences in the respective characteristics of the responses mediated by the two α-adrenoceptor subtypes. α_1-Adrenoceptor agonists elicit responses which are rapid in onset, short-lasting and appear to utilize intracellular calcium; α_2-adrenoceptor agonists elicit responses that are slower in onset, longer lasting and appear to utilize extracellular calcium which enters the cell through voltage-dependent channels (Langer & Shepperson, 1981; McGrath, 1982; Cavero *et al.*, 1983).

Only very recently has the existence of smooth muscle α_2-adrenoceptors been demonstrated in a peripheral, well-innervated muscular artery *in vitro*, using the rat isolated tail artery preparation (Medgett & Langer, 1984; Hicks *et al*, 1984). The presence of both α_1- and α_2-subtypes on smooth muscle cells in rat tail arteries was reported in the latter studies, based on the effects of idazoxan (selective α_2-adrenoceptor antagonist; Doxey *et al.*, 1983) and prazosin (selective α_1-adrenoceptor antagonist) on responses to NA and to TL99 (selective α_2-adrenoceptor agonist; Hicks & Cannon, 1981). In addition, the rat isolated tail artery is a stable and sensitive preparation with some of the characteristics of a true resistance vessel (Cheung, 1982a,b) and therefore, is particularly suitable for *in vitro* studies.

In previous studies (Medgett & Langer, 1984; Hicks *et al.*, 1984) data were obtained under conditions of blockade of β-adrenoceptors and of inhibition of neuronal uptake using propranolol and cocaine, respectively. In the present study it was considered important to confirm that effects of selective α-adrenoceptor antagonists, as observed in the above studies, were qualitatively similar in the *absence* of blockade of β-adrenoceptors and neuronal uptake, since data from *in vivo* studies were obtained in the absence of these agents. The primary aim of the present study, however, was to investigate the dependence of α-adrenoceptor-mediated responses on extracellular calcium ion concentration by lowering the concentration of calcium ions in the perfusate and also by assessing the effects of NA and sympathetic nerve stimulation in the presence of the calcium slow channel antagonist, diltiazem. Some of these data have been published in abstract form (Rajanayagam & Medgett, 1984a, b).

Methods

Preparation of rat tail artery

Male Sprague-Dawley rats weighing 200 to 300 g were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}) administered intraperitoneally. A proximal segment, approximately 1 cm in length, of the tail artery was removed and placed in a dish of Krebs solution. The vessel was cannulated at the proximal end by use of a dissecting microscope. The distal end was tied and a large opening made just proximal to the tie. The artery was then mounted vertically, distal end uppermost, under a tension of 0.5 g for perfusion and superfusion. Two circular platinum electrodes, separated by 4 mm were located around the proximal end of the artery segment to allow field stimulation of the adventitial sympathetic nerves (sympathetic nerve stimulation, SNS). The artery was perfused through the bottom cannula at a flow rate of 4 ml min^{-1} (LKG peristaltic pump) with Krebs solution. The solution perfused the lumen and then passed out through the opening at the distal end to superfuse the extraluminal surface. The Krebs solution in the supply reservoir was continually gassed with a mixture of 5% CO_2 in O_2 and was maintained at 37 °C.

Vasoconstriction was measured as increases in perfusion pressure under conditions of constant flow, using a pressure transducer connected to a Rikadenki potentiometric pen recorder with a chart speed of 0.5 cm min^{-1}. A 10 to 15 min stabilization period was allowed before any responses to stimulation or α-adrenoceptor agonists were elicited. During this period the resting perfusion pressure fell from an initial level of 100 to 200 mmHg to 20 to 40 mmHg and thereafter remained relatively constant. After the stabilization period, a few responses were elicited to SNS to determine the viability of the preparation. The artery segment was considered to be viable if maximal responses of 40 mmHg or more could be elicited with SNS at frequencies of 1 to 3 Hz. After perfusion/superfusion for a further 20 min, responses to SNS and NA were obtained as indicated below.

Stimulation of adventitial sympathetic nerves

Electrical stimulation consisted of monophasic square wave pulses of 0.3 ms duration delivered at supramaximal voltage. Responses were elicited successively to stimulation at frequencies of 0.1, 0.3, 1, 3 and 10 Hz. For each frequency stimulation was continued until the maximum increase in perfusion pressure had been obtained (5–20 s).

Concentration-response relationships to noradrenaline

Solutions containing NA were given by momentarily stopping perfusion and replacing the Krebs solution with one containing the required concentration of the agonist, which was subsequently perfused until the maximum response was obtained, before returning to drug-free Krebs solution (1–2 min).

Experimental protocol

An initial frequency-response curve to SNS which was not considered in the statistical analysis of the data was obtained to allow the preparation to stabilize. After a 25 min period the next frequency-response curve obtained was counted as the first. This was followed after a further 25 min by a non-cumulative concentration-response curve to NA using stepwise 3 fold increments in concentration. (Only one agonist was used in each experiment). To avoid desensitization of the artery, responses of no greater than 200 mmHg were obtained in the first concentration-response curve. After an interval of 25 min the second frequency-response curve was obtained and was followed, 25 min later, by a second concentration-response curve in which the concentration of NA producing the maximum response was established.

The effects of antagonists were assessed by adding them to the Krebs solution, in the required concentration, immediately after establishing the first agonist concentration-response curve.

The effects of a decreased concentration of extracellular calcium ions on responses to NA and SNS were assessed by decreasing the concentration of calcium in the Krebs solution from 2.5 mmol l^{-1} to 0.6 mmol l^{-1} immediately after establishing the first agonist concentration-response curve.

Composition of Krebs solution

The Krebs solution was of the following composition (mmol l^{-1}): NaCl 118, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 0.45, KH_2PO_4 1.03, $CaCl_2$ 2.5, D-(+)-glucose, 11.1, sodium ethylenediamine tetraacetic acid (EDTA) 0.067. The pH of the Krebs solution was 7.4–7.6.

Drugs

Drugs used were: noradrenaline hydrochloride (Sigma); idazoxan hydrochloride (RX781094, Reckitt & Colman); prazosin hydrochloride (Pfizer); diltiazem hydrochloride (L.E.R.S. Synthelabo). The salts, except prazosin hydrochloride, were dissolved in distilled water which, in the case of NA, contained ascorbic acid 0.05 mg ml^{-1}. Prazosin was suspended in 5 ml glycerol before making up the volume of a 1 mmol l^{-1} stock solution with 5% dextrose.

Statistical analysis

Results are expressed as the mean ± s.e. Statistical analysis was performed using Student's *t* test, probability levels less than 0.05, for paired or unpaired data, as appropriate. The dissociation constants (K_B) of the antagonists were determined according to the formula (Furchgott, 1972):

$$K_B = \frac{[\text{antagonist}]\ M}{\text{concentration ratio} - 1}$$

The concentration ratio is the ratio, determined in a single experiment, of the concentrations of NA giving an equal response in the presence or in the absence of the antagonist; the response is measured as the EC_{50} value where lines do not depart significantly from parallelism or linearity. For lines that do depart from parallelism or linearity, the dissociation constants are determined from responses measured at the EC_{25} and EC_{75} levels, as well as the EC_{50} level.

Results

Control responses of preparations of rat tail arteries to NA (30–300 nmol l^{-1}) and to SNS (0.1–10 Hz) are shown in Figure 1. For each concentration the agon-

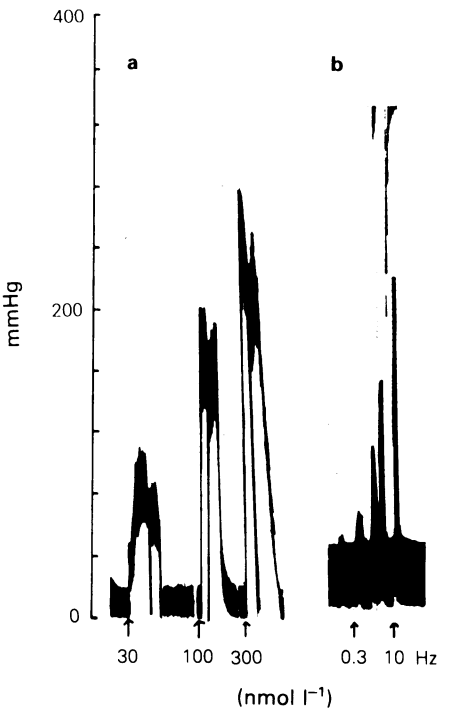

Figure 1 (a) Effects of noradrenaline (NA) in rat isolated perfused tail artery. Ordinate scale: perfusion pressure in mmHg. Control responses to concentrations of NA between 30 and 300 nmol l^{-1} are shown. (b) Effects of sympathetic nerve stimulation in rat isolated perfused tail artery. Ordinate scale: Perfusion pressure in mmHg. Control responses to frequencies between 0.1 and 10 Hz are shown. Frequencies of 0.3 and 10 Hz are indicated by arrows.

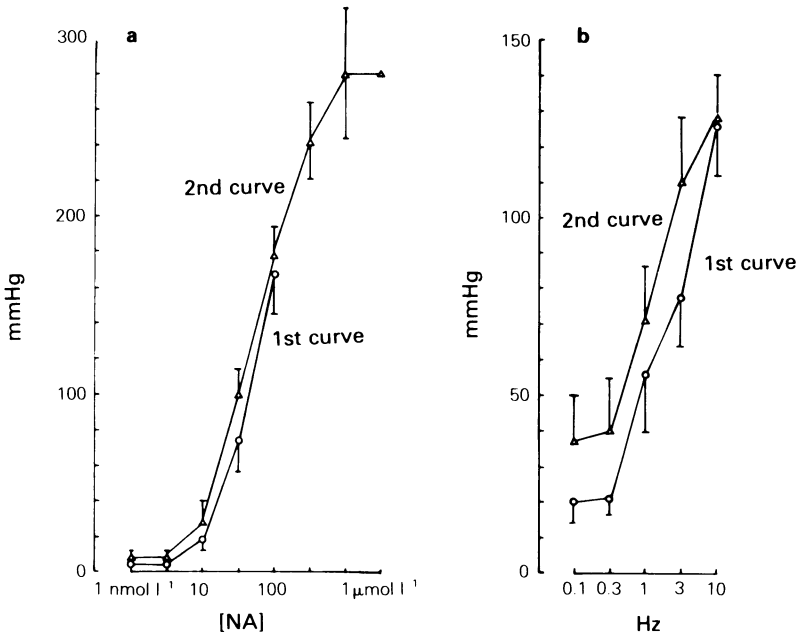

Figure 2 (a) Control concentration-response curves to noradrenaline (NA) in rat isolated perfused tail arteries. Ordinate scale: response in mmHg; abcissa scale: log molar concentration of NA. Mean values and s.e. are given for NA ($n=7$). (b) Control frequency-response curves to sympathetic nerve stimulation in rat isolated perfused tail arteries. Ordinate scale: Response in mmHg. Abscissa scale: Frequency of stimulation in Hz. Mean values and s.e. are given ($n=13$).

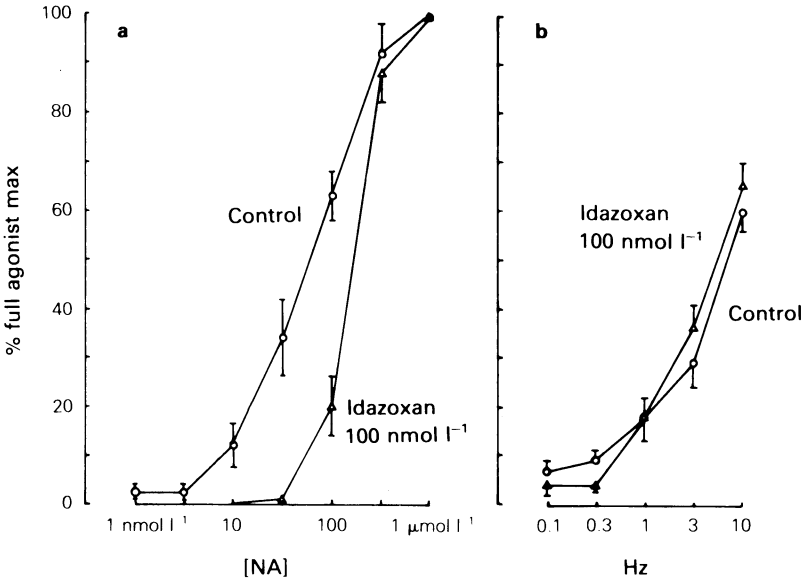

Figure 3 (a) Effects of idazoxan (100 nmol l^{-1}) on responses to noradrenaline (NA) in rat isolated perfused tail arteries. Ordinate scale: peak contractile response expressed as a percentage of the maximal response to full agonist. Abscissa scale: log molar concentration of NA. Mean values and s.e. are given for NA in the presence and absence of idazoxan ($n=7$). The curves depart significantly ($P<0.05$) from parallelism. (b) Effects of idazoxan (100 nmol l^{-1}) on frequency-response curves to SNS in rat isolated perfused tail arteries. Ordinate scale: Peak contractile response expressed as a percentage of the maximum response to full agonist. Abscissa scale: frequency of stimulation in Hz. Mean values and s.e. are given for responses to SNS in the presence and absence of idazoxan ($n=13$).

ists were infused until the maximum responses were obtained (1–2 min). Similarly, stimulation at each frequency was applied until the maximum response was obtained (3–5 s).

Figure 2a shows first and second concentration-response curves to NA based on pooled data from all control experiments. Responses of no greater than 200 mmHg were elicited in the first concentration-response curve in each preparation. For the second concentration-response curve, the concentrations of NA were progressively increased up to those producing maximum responses.

The linear portions of the first and second concentration-response curves for NA did not depart significantly from coincidence ($P > 0.05$). The mean pD_2 value for NA, determined from the first concentration-response curve was 7.20 (s.e. = 0.11, $n = 7$) and that determined from the second concentration-response curve was 7.30 (s.e. = 0.09, $n = 7$). In addition, responses to NA were highly reproducible from one preparation to another.

In order to assess the effects of antagonists on the NA concentration-response relationships, comparisons were thus made between the second curve constructed from the pooled data from control experiments, and the second curve constructed from the pooled data from experiments in which the antagonist was introduced 60 min before commencing the second curve.

Figure 2b shows the first and second frequency-response relationships to field SNS pooled from the data from all control experiments. There was no significant departure from coincidence between the linear portions of the two frequency-response curves. Due to the fairly substantial between-preparation variation in these responses, comparisons were made using paired t tests with the first and second frequency-response curves when assessing the effects of antagonists which were present during the second curves.

Effects of idazoxan

Idazoxan (100 nmol l^{-1}), when added to the perfusion solution, did not cause any change in the resting perfusion pressure. However, idazoxan (100 nmol l^{-1}) displaced the NA concentration-response curve to the right (Figure 3a). The NA curve departed significantly from parallelism with the control curve ($P < 0.05$) and therefore log shift values were determined at EC_{25}, EC_{50} and EC_{75} levels of the curve. The displacement of the concentration-response curve was greater with low concentrations than with high concentrations of NA: thus, Table 1 shows that at the EC_{25} level the log shift was 0.60 log units (s.e. = 0.07, $n = 7$) whereas at the EC_{75} level the log shift was only 0.24 log units (s.e. = 0.10, $n = 7$). Idazoxan had no effect on the maximum response elicited by NA. Idazoxan (100 nmol l^{-1}) had no effect on responses to SNS at frequencies of 1Hz and above; of those preparations responding to the lower frequencies of 0.1 and 0.3 Hz, all gave a decreased response after idazoxan (Figure 3b).

Effects of prazosin

Prazosin (10 nmol l^{-1}), when added to the perfusion solution, did not cause any change in the resting perfusion pressure. Prazosin displaced the NA concentration-response curve to the right by 1.72 log units (s.e. = 0.70, $n = 6$; Table 1). In the presence of prazosin, the concentration-response curve to NA did not depart significantly from linearity or from parallelism with the control curve (Figure 4a). Prazosin did not significantly alter the maximum response elicited by NA.

In contrast to the lack of effect of idazoxan on responses to SNS, prazosin significantly and markedly reduced these responses (Figure 4b).

Table 1 Antagonism of responses to noradrenaline (NA) by idazoxan (100 nmol l^{-1}) and prazosin (10 nmol l^{-1}) in rat isolated perfused tail arteries.

			Idazoxan			*Prazosin*		
	NA control	n	*Log shift*	$-\log K_B$	*n*	*log shift*	$-\log K_B$	*n*
$-\log EC_{25}$	7.64 (0.12)	7	0.60 (0.07)	7.47	7	1.76 (0.08)	9.75	6
$-\log EC_{50}$ (pD_2)	7.30 (0.09)	7	0.48 (0.10)	7.31	7	1.72 (0.70)	9.71	6
$-\log EC_{75}$	6.79 (0.14)	7	0.24 (0.01)	6.87	7	1.69 (0.11)	9.68	6

Significant ($P < 0.05$) shifts of curves were obtained in all cases at EC_{25}, EC_{50} and EC_{75} levels with both idazoxan (100 nmol/l) and prazosin (10 nmol/l).
Values in parentheses indicate s.e.

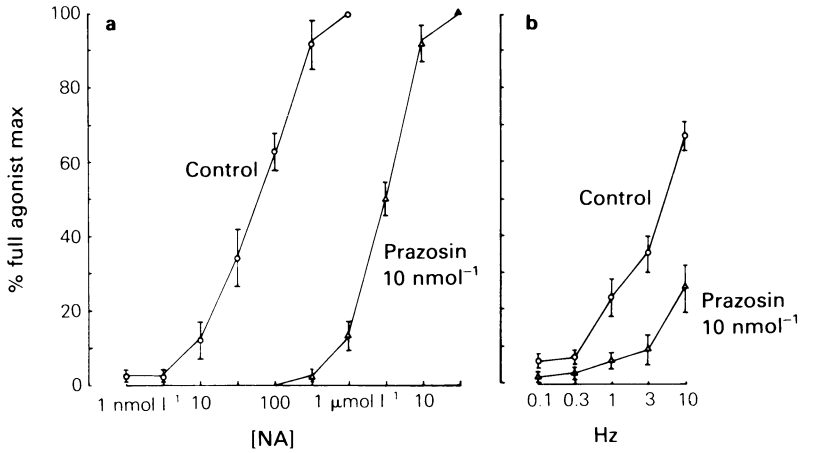

Figure 4 (a) Effects of prazosin (10 nmol l^{-1}) on concentration-response curves to noradrenaline (NA) in rat isolated perfused tail arteries. Ordinate scale: peak contractile response expressed as a percentage of the maximum response to full agonist. Abscissa scale: log molar concentration of NA. Mean values and s.e. are given for NA in the presence and absence of prazosin (n= 6). (b) Effects of prazosin (10 nmol l^{-1}) or frequency-response curves to SNS in rat isolated perfused tail arteries. Ordinate scale: Peak contractile response expressed as a percentage of the maximum response to full agonist. Abscissa scale: frequency of stimulation in Hz. Mean values and s.e. are given for responses to SNS in the presence and absence of prazosin

Effects of lowering the concentration of calcium ions

When the concentration of calcium ions in the Krebs solution was reduced from 2.5 mmol l^{-1} to 0.6 mmol l^{-1}, the concentration-response curve to NA was significantly displaced to the right by 0.44 log units (s.e. = 0.04, n = 6; Table 2). There was no significant departure of the concentration-response curve which was obtained after reducing the concentration of calcium ions either from linearity or from parallelism with the control curve. There was no significant effect on the maximum response elicited by NA (Figure 5a).

Responses to SNS were not significantly affected by the reduction of the concentration of calcium ions in the Krebs solution (Figure 5b).

Effects of diltiazem

Diltiazem (1 and 10 μmol l^{-1}), when added to the perfusion solution, did not cause any change in the resting perfusion pressure. The concentration-

Table 2 Effects of lowering the concentration of calcium ions in the Krebs solution and of diltiazem on responses to noradrenaline (NA) in rat isolated perfused tail arteries

	pD_2	*Maximum response* (mmHg)	*log shift*	n
NA control ($[Ca^{2+}]$ = 2.5 mmol l^{-1})	7.30 (0.09)	280 (32)	–	7
$[Ca^{2+}]$ = 0.6 mmol l^{-1} [diltiazem] = 0	6.81 (0.01)	258 (27)	0.44 (0.04)*	6
$[Ca^{2+}]$ = 2.5 mmol l^{-1} [diltiazem] = 1 μmol l^{-1}	6.57 (0.05)	240 (20)	0.73 (1.06)*	6
$[Ca^{2+}]$ = 2.5 mmol l^{-1} [diltiazem] = 10 μmol l^{-1}	6.30 (0.06)	238 (20)	0.90 (0.08)*	7

S.e. mean shown in parentheses.
* Significant (P<0.05) rightward shift.

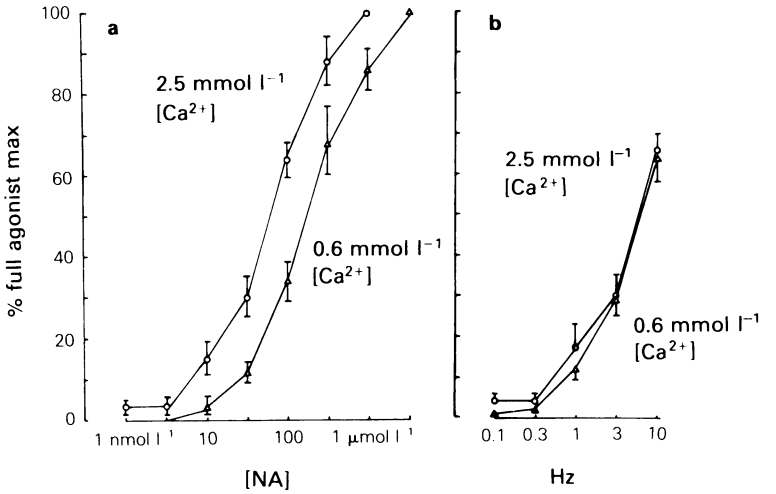

Figure 5 (a) Effects of lowering the concentration of calcium ions in the Krebs solution from 2.5 mmol l^{-1} to 0.6 mmol l^{-1} on concentration-response curves to noradrenaline (NA) in rat isolated perfused tail arteries Ordinate scale: peak contractile response expressed as a percentage of the maximum response to full agonist Abscissa scale: log molar concentration NA. Mean values and s.e. are given for responses to NA before and after reducing the concentration of calcium ions in the Krebs solution ($n = 6$). (b) Effects of lowering the concentration of calcium ions in the Krebs solution from 2.5 to 0.6 mmol l^{-1} on frequency-response curves to SNS in rat isolated perfused tail arteries. Ordinate scale: peak contractile response expressed as a percentage of the maximum response to full agonist. Abscissa scale: frequency of stimulation in Hz. Mean values and s.e. are given for responses to SNS before and after the concentration of calcium ions in the Krebs solution was reduced ($n = 12$).

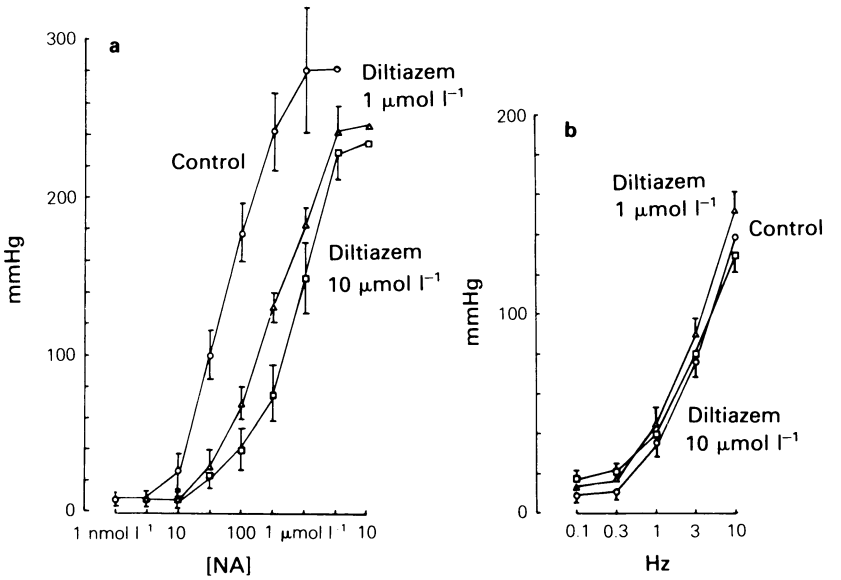

Figure 6 (a) Effects of diltiazem (1 and 10 μmol l^{-1}) on concentration-response curves to noradrenaline (NA) in rat isolated perfused tail arteries. Ordinate scale: peak contractile response in mmHg. Abscissa scale: log molar concentration of NA. Mean values and s.e. are given for responses to NA in the presence and absence of diltiazem ($n = 7$). (b) Effects of diltiazem (1 and 10 μmol l^{-1}) on frequency-response curves to SNS in rat isolated perfused tail arteries. Ordinate scale: peak contractile responses in mmHg. Abscissa scale: frequency of stimulation in Hz. Mean values and s.e. are given for responses to SNS in the presence and absence of diltiazem ($n = 13$).

response curve to NA, obtained in the presence of diltiazem (1 μmol l^{-1}) did not significantly depart either from linearity or from parallelism with the control curve. Diltiazem tended to reduce the maximum response elicited by NA (Figure 6a); thus log shift values were calculated at the EC_{50} level of the control curve. Diltiazem (1 μmol l^{-1}) significantly displaced the NA curve to the right by 0.73 log units (s.e. = 0.06, $n = 6$; Table 2). A higher concentration of ditiazem (10 μmol l^{-1}) further displaced the curve to the right by 0.17 log units. The total displacement of the concentration-response curve to NA, obtained in the presence of diltiazem (10 μmol l^{-1}) from the control curve was thus 0.90 log unit (s.e. = 0.08, $n = 7$; Table 2).

Responses to SNS were not significantly affected by either concentration of diltiazem (Figure 6b).

Discussion

Smooth muscle α_2-adrenoceptors have been identified previously in proximal segments of tail arteries of SHR and Sprague-Dawley rats, by use of the agonists NA, methoxamine and TL99 in combination with the antagonists idazoxan, prazosin and corynanthine (see Hicks *et al.*, 1984; Medgett & Langer, 1984; Medgett *et al.*, 1984). The most direct evidence for the presence of smooth muscle α_2-adrenoceptors was the effect of the selective α_2-adrenoceptor agonist TL99 (Hicks & Cannon, 1981): in SHR tail arteries, responses to TL99 were substantially reduced by idazoxan (100 nmol l^{-1}), unaffected by prazosin (1 nmol l^{-1}) and virtually abolished if the extracellular calcium concentration was halved (Hicks *et al.*, 1984). In contrast, responses to the selective α_1-adrenoceptor agonist methoxamine were unaffected by idazoxan, markedly reduced by prazosin and unaffected by halving the calcium concentration (Hicks *et al.*, 1984); thus both α_1- and α_2-adrenoceptors would appear to be present on smooth muscle cells in this vessel. In both SHR and Sprague-Dawley rat tail arteries, the non-selective agonist NA activates both α-adrenoceptor subtypes since both idazoxan and prazosin reduce responses in the appropriate selective concentrations (Medgett & Langer, 1984; Medgett *et al.*, 1984). All data referred to above were obtained under conditions of blockade of β-adrenoceptors and of inhibition of neuronal uptake using propranolol and cocaine, respectively.

In the present study it was intended to confirm that positive evidence for the existence of smooth muscle α_2-adrenoceptors could also be obtained, using NA as an agonist, in the absence of blockade of β-adrenoceptors and neuronal uptake; this latter situation applies to the bulk of the available *in vivo* data (see McGrath, 1982). The dependence of α-adrenoceptor-mediated vasoconstrictor responses on the concentration of extracellular calcium ions and the effect of the calcium slow channel blocker diltiazem were also investigated.

Vasoconstrictor responses elicited by NA were antagonized by a selective α_2-adrenoceptor antagonist concentration of idazoxan (100 nmol l^{-1}); this is ten times lower than that required for minimal α_1-adrenoceptor blocking actions (Doxey *et al.*, 1983). The $-\log K_B$ values, determined at the EC_{25}, EC_{50} and EC_{75} levels approach those values quoted for α_2-antagonist activity (Doxey *et al.*, 1983), confirming that α_2-adrenoceptors contributed to the vasoconstrictor responses elicited by NA. Idazoxan (100 nmol l^{-1}) was particularly effective against concentrations of NA between 1 and 100 nmol l^{-1}; the log shift value determined at the EC_{25} level of the curve was significantly greater than that determined at the EC_{75} level of the curve. This suggests the possibility that in rat isolated tail arteries, low concentrations of NA (of 1–100 nmol l^{-1}) activate α_2-adrenoceptors to a greater extent than do higher concentrations of NA; this is similar to *in vivo* findings (see McGrath, 1982). The observation that idazoxan causes a 10 fold increase in the threshold concentrations of NA but has no effect on the concentration required to elicit the maximum response further supports the above-mentioned suggestions. It would appear that this pattern of antagonism by idazoxan may be dependent on the absence of blockade of neuronal uptake and/or β-adrenoceptors, since parallel shifts of the NA curve were obtained with this concentration of idazoxan in the presence of cocaine and propranolol (Medgett & Langer, 1984).

The responses to SNS have been shown to be almost completely abolished by tetrodotoxin, confirming their neurogenic origin (Medgett & Langer, 1984). Therefore the lack of effect of idazoxan on these responses suggests that endogenous NA, released from nerve terminals, does not activate postjunctional α_2-adrenoceptors. It may be argued that blockade of prejunctional release-modulating α_2-adrenoceptors, which have previously been identified in this tissue (Medgett & Rand, 1981) increases the release of NA from sympathetic nerve terminals and that this counteracts the effect of idazoxan on postjunctional α_2-adrenoceptors which may be present. However, at 1 Hz, the feedback mechanism operates to only a small degree (Medgett & Rand, 1981), yet idazoxan has no effect on the response at this frequency. Some involvement of postjunctional α_2-adrenoceptors may not be completely excluded at the very lowest frequencies used (0.1 and 0.3 Hz), although their role, if it exists, is insignificant compared to that in SHR arteries, where a 10 fold lower concentration of idazoxan markedly reduced re-

sponses up to 3 Hz under identical experimental conditions (Medgett *et al.*, 1984). Notwithstanding the existence of smooth muscle α_2-adrenoceptors, the co-existence of α_1-adrenoceptors as the predominant subtype in rat tail arteries (Medgett & Langer, 1984) has also been confirmed in the present study by the potent effect of prazosin on responses to NA. The $-\log K_B$ values, determined at the EC_{25}, EC_{50} and EC_{75} levels of the NA curve were not significantly different. The $-\log K_B$ values obtained in the presence of prazosin approach 10. In addition, the sensitivity to blockade by prazosin of responses to SNS, compared with the resistance of these responses to idazoxan, further adds to the evidence indicating a preferential activation of α_1-adrenoceptors by neuronally released NA (see McGrath, 1982; Medgett & Langer, 1984).

In order to investigate the dependence of α-adrenoceptor-mediated responses on extracellular calcium, the concentration of calcium ions in the Krebs solution was reduced from 2.5 to 0.6 mmol l^{-1}. This significantly reduced responses to NA at all levels of the curve: there was a clear increase in the threshold concentration of NA as well as an increase in the concentration of NA required to elicit the maximum response. In a few experiments (unpublished data) the concentration of calcium ions was further reduced to 0.25 mmol l^{-1}; there was no further reduction of the responses to NA. It is possible that the component inhibited by reducing extracellular calcium corresponds to the α_2-adrenoceptor-mediated component, as revealed by the antagonistic effects of idazoxan. This possibility gains support from the apparent lack of effect of reducing the concentration of calcium ions on responses to SNS, since these latter responses appear to be mediated almost exclusively by α_1-adrenoceptors. Similarly, the results obtained with diltiazem support the above postulation: responses to NA were substantially reduced whereas those to SNS were unaffected. It is interesting that increasing the concentration of diltiazem from 1 to 10 μmol l^{-1} caused little further antagonism of NA responses. Higher diltiazem concentrations were not tested since these cause appreciable direct α_1-adrenoceptor antagonism rather than simply blocking calcium channels (Nayler *et al.*, 1982; Cavero *et al.*, 1983). The effect of 10 μmol l^{-1} diltiazem thus probably represents a near maximal effect of blockade of entry of extracellular calcium ion on responses to NA in this vessel, and amounts to 0.9 log unit shift in the NA curve. That this corresponds closely to the maximal shift attributable to blockade of α_2-adrenoceptors by idazoxan, in the presence of cocaine and propranolol, of about 0.8 of a log unit (Medgett & Langer, 1984), gives further weight to the proposed correlation between α_2-adrenoceptor-mediated and extracellular calcium sensitive components of responses to NA. Experiments reassessing the effects of the antagonists idazoxan and prazosin were not performed, since it has been shown in other experiments (unpublished data) that the antagonistic effect of 100 nmol l^{-1} idazoxan is lost if the NA curve is previously shifted about one log unit to the right by prazosin. In other words, higher NA concentrations appear to lose selectivity for α_2-adrenoceptors, and thus any interpretation of the effects of idazoxan on NA in reduced calcium would have to account for this factor.

In conclusion, the effects of idazoxan and prazosin on responses to NA and SNS confirm previous data (Medgett & Langer, 1984; Hicks *et al.*, 1984), suggesting that α_1-adrenoceptors predominate in proximal segments of rat tail artery, but that there exists a subpopulation of smooth muscle α_2-adrenoceptors; in the present instance, neuronal uptake and β-adrenoceptors were not blocked. Exogenous agonists activate both α_1-and α_2-subtypes whereas NA released from nerve terminals almost exclusively activates α_1-adrenoceptors; this may be attributed to a hypothetical differential intramural distribution of α_1- and α_2-adrenoceptors in arterial smooth muscle (see Introduction). Finally, it is proposed that α_2-adrenoceptor-, but not α_1-adrenoceptor-mediated responses are dependent on extracellular calcium. This is indicated by the apparent correlation between the α_2-adrenoceptor-mediated component of NA responses, and their sensitivity, either to changes in the concentration of calcium ions in the Krebs solution or to blockade by diltiazem in contrast to the resistance of responses to SNS. These results confirm previously reported *in vivo* and *in vitro* data using isolated venous smooth muscle (Cavero *et al.*, 1983).

The work described in this paper was supported by the National Health and Medical Research Council of Australia.

References

CAVERO, I., SHEPPERSON, F., LEVÈVRE-BORG, F. & LANGER, S.Z. (1983). Differential inhibition of vascular smooth muscle responses to α_1- and α_2-adrenoceptor agonists by diltiazem and verapamil. *Circulation Res.*, **52** (Suppl. 1), 69–76.

CHEUNG, D.W. (1982a). Spontaneous and evoked excitat-

ory junction potentials in rat tail arteries. J. Physiol., **328,** 449–459.

CHEUNG, D.W. (1982b). Two components in the cellular responses of rat tail arteries to nerve stimulation. *J. Physiol.*, **328,** 461–468.

DOCHERTY, J.R. & McGRATH, J.C. (1980a). A comparison of pre- and postjunctional potencies of several alpha-adrenoceptor agonists in the cardiovascular system and anococcygeus muscle of the rat. *Naunyn-Schmiedebergs Arch. Pharmac.*, **312,** 107–116.

DOCHERTY, J.R. & McGRATH, J.C. (1980b). An examination of factors influencing adrenergic transmission in pithed rat, with special reference to noradrenaline uptake. *Naunyn-Schmiedebergs Arch. Pharmac.*, **313,** 101–111.

DOXEY, J.C., ROACH, A.G. & SMITH, C.F.C. (1983). Studies on RX781094, a new selective potent specific antagonist of α_2-adrenoceptors. *Br. J. Pharmac.*, **78,** 489–506.

DREW, G.M. & WHITING, S.B. (1979). Evidence for two distinct types of post-synaptic α-adrenoceptor in vascular smooth muscle *in vivo*. *Br. J. Pharmac.*, **67,** 207–215.

FLAVAHAN, N.A. & McGRATH, J.C. (1980). Blockade by yohimbine of prazosin-resistant effects of adrenaline in the pithed rat. *Br. J. Pharmac.*, **69,** 355–357.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Catecholamines*, Handb. exp. Pharmac., N.S. Vol. 33. ed. Blaschko, H. & Muscholl, E. pp. 283–335, Berlin and Heidelberg: Springer-Verlag.

HAMILTON, C.A. & REID, J.L. (1980). Postsynaptic location of α_2-adrenoceptors in vascular smooth muscle. *Br. J. Pharmac.*, **70,** 63–64P.

HICKS, P.E. & CANNON, J.G. (1981). Cardiovascular effects of 2-(N, N-dimethyl)-amino-6, 7-dihydroxy-1, 2, 3, 4, tetrahydronaphthalene in the pithed rat. Differential antagonism by yohimbine and prazosin. *J. Pharm. Pharmac.*, **32,** 786–789.

HICKS, P.E., MEDGETT, I.C. & LANGER, S.Z. (1984). Postsynaptic α_2-adrenergic receptor mediated vasoconstriction in SHR tail arteries *in vivo*. *Hypertension*, **6,** I–12 – I–18.

LANGER, S.Z. (1974). Presynaptic regulation of catecholamine release. *Biochem. Pharmac.*, **23,** 1793–1800.

LANGER, S.Z. & SHEPPERSON, N.B. (1981). Antagonism of alpha-adrenoceptor mediated contractions of the isolated saphenous vein of the dog by diltiazem and verapamil. *Br. J. Pharmac.*, 942–943P.

LANGER. S.Z. & SHEPPERSON, N.B. (1982). Recent developments in vascular smooth muscle pharmacology-the postsynaptic α_2-adrenoceptor. *TIPS*, **3,** 1.112–1.118.

McGRATH, J.C. (1982). Evidence for more than one type of postjunctional α-adrenoceptor. *Biochem. Pharmac.*, **31,** 467–484.

MEDGETT, I.C., HICKS, P.E. & LANGER, S.Z. (1984). Smooth muscle alpha$_2$-adrenoceptors mediate vasoconstrictor responses to exogenous norepinephrine and to sympathetic stimulation to a greater extent in SHR than in WKY rat tail arteries. *J. Pharmac. exp. Ther.*, **229,** 823–830.

MEDGETT, I.C. & LANGER, S.Z. (1984). Heterogeneity of smooth muscle α-adrenoceptors in rat tail artery *in vitro*. *J. Pharmac. exp. Ther*, (in press).

MEDGETT, I.C. & RAND, M.J. (1981). Dual effects of clonidine on rat prejunctional α-adrenoceptors. *Clin. exp. Pharmac. Physiol.*, **8,** 503–507.

NAYLER, W.G., THOMPSON, J.E. & JARROT, B. (1982). The interaction of calcium antagonists (slow channel blockers) with myocardial alpha adrenoceptors. *J. Mol. Cell. Cardiol.*, **14,** 185–188.

RAJANAYAGAM, M.A.S. & MEDGETT, I.C. (1984a). Dependence of α-adrenoceptor-mediated vasoconstrictor responses in rat tail artery on extracellular calcium ion concentration. *Clin. exp. Physiol. Pharmac.*, Suppl. **8,** 54.

RAJANAYAGAM, M.A.S. & MEDGETT, I.C. (1984b). Effect of diltiazem on α-adrenoceptor-mediated vasoconstrictor responses in rat tail artery. *Proc. IXth Int. Congr. Pharmac.*, Abstract 1648.

(*Received April 18, 1984. Revised June 18, 1984.*)